240. Studies on Haptoglobin. III

Physicochemical Properties of Hemoglobin-Haptoglobin Complex and Hemoglobin-Antihuman Hemoglobin Antibody Complex. I

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Introduction. Although similarities in molecular structure between haptoglobin (Hp) and antibody were reported,¹⁾⁻³⁾ many differences in immunochemical properties between these two molecules were also clarified.^{4),5)} Immunological precipitation reaction and complement fixation reaction observed in hemoglobin antihemoglobin antibody interaction were not seen in hemoglobin haptoglobin interaction.⁴⁾ These immunological differences between hemoglobin-haptoglobin complex (Hb-Hp complex) and hemoglobin-antihemoglobin antibody complex (Hb-Ab complex) lead to the concept that higher structures of Hb bound by Hp and that of Hb bound by antihemoglobin antibody are different from each other. In this communication, differences in higher structure between Hb-Hp complex and Hb-Ab complex were discussed from the data on circular dichroism spectra (CD spectra) of these two complexes.

Materials and methods. *Preparation of Hb-Hp 2-1 complex.* Hb-Hp 2-1 complex was prepared by the method of Hamaguchi²⁾ with some modification in column size. The purified complex was passed through Sephadex G-25 column equilibrated with 0.05 M sodium phosphate buffer, pH 7.0.

Purification of soluble Hb-Ab complex. Specific antibody to Hb A₁ was purified by using immunoabsorbent described by Avrameas and Ternynck.⁶⁾ The purified antihemoglobin antibody was added to an excess of purified Hb A₁ to form soluble Hb-Ab complex. This clear solution was concentrated to 10 ml by evacuating in a collodion bag and Hb-Ab complex was separated from free Hb A₁ by gel filtration through Sepharose 6 B column, 1.2×70 cm, equilibrated with 0.05 M sodium phosphate buffer, pH 7.0.

CD spectra of Hb, Hb-Hp complex, and Hb-Ab complex. CD measurements were performed at 25° using a Jouan Dichrograph II.

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The heme concentration of these three samples was 50–150 μ M. Molar ellipticities are given on a heme basis.

Results and discussion. Figure 1 shows the visible CD spectra of oxy Hb-Hp complex, oxy Hb A, and oxy Hb-Ab complex. A trough near 520 $m\mu$ is positive for Hb-Hp complex whereas it is negative for Hb A and for Hb-Ab complex. And the negative of the trough is greater for Hb-Ab complex than for Hb A. Intensity ratio of the positive band at 578 $m\mu$ and at 546 $m\mu$ is greater for Hb-Hp complex than for Hb-Ab complex, and the greatest for Hb A. In Fig. 2, CD spectra of these oxygenated compounds in a region from 240 to 450 $m\mu$ are shown. Just as seen in the region at 520 $m\mu$, negative

Fig. 1. The visible circular dichroism spectra of Hb A, Hb-Hp complex, and Hb-Ab complex in 0.05 M sodium phosphate buffer, pH 7.0, at 25°.

Fig. 2. The circular dichroism spectra of Hb A, Hb-Hp complex, and Hb-Ab complex between 240 $m\mu$ and 450 $m\mu$ in 0.05 M sodium phosphate buffer, pH 7.0 at 25°.

troughs are observed for both Hb A and Hb-Ab complex in the 400 $m\mu$ region and the corresponding trough for Hb-Hp complex is positive. Intensity of the prominent positive band at 420 $m\mu$ seems to be similar among three compounds in these conditions. A negative trough around 280 $m\mu$ seen in Hb-Hp complex is thought to be a simple effect of Hp which has a negative trough at around 280 $m\mu$.⁷⁾ The same negative trough just as seen in Hb-Hp complex but not in Hb A is observed in Hb-Ab complex at around 280 $m\mu$. The principal band at 260 $m\mu$ can be resolved into three positive bands for Hb A, and into two positive bands for both Hb-Hp complex and Hb-Ab complex.

From these observations, differences in environmental structure around the heme between Hb-Ab complex and Hb-Hp complex are clarified which may have some connection with the differences in immunochemical reactions between these two complexes.

Summary. Soluble hemoglobin-antihemoglobin antibody complex (Hb-Ab complex) was purified. Circular dichroism spectra of Hb A, Hb-Hp complex, and Hb-Ab complex were measured and the differences in higher structure of these two complexes were discussed

in connection with the differences in their immunochemical properties.

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